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COMMUNICATION REGARDING
EXTENSION OF TIME LIMIT

(PCT Rule 26.2)

Date of mailing *(day/month/year)* 30 JUL 1999	
Applicant's or agent's file reference 1102865-0034	**IMPORTANT COMMUNICATION**
International application No. PCT/US99/10750	International filing date *(day/month/year)* 14 MAY 99
Applicant APHTON CORPORATION	

1. In response to the applicant's request of 16 Jun 99, the time limit for replying to:

   [X] the Invitation to Correct Defects (PCT/RO/106)

   [ ] (other) _____

   has been extended as follows:

   [X] extension of 1 months days from 04 Jul 99

   [X] extension until 04 Aug 99

2. [ ] No extension of the time limit is granted and the time limit remains as previously set.

Name and mailing address of the receiving Office Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Authorized officer Tredelle Jackson Telephone No. 703-305-3165

Form PCT/RO/138 (July 1992)

colorectal cancers, and, in particular, increased amounts of the hormone precursor progastrin have been detected in many colorectal tumors using gastrin antisera (Ciccotosto et al.1995). More recently,it has been discovered that many of these cancer cells also secrete gastrin and thus effect an autonomous proliferative pathway(Van-Solinge et al.1993,Nemeth et al.1993 and Seva et al. 1994).

The peptide hormones G17 and G34 bind to the CCK-B/gastrin receptors on the cell membranes of normal cells.However, it has been found that G17,but not G34, stimulates the growth of gastrin-dependent cancer cells.Serum-associated G17,in particular,has the potential to stimulate the growth of colorectal tumors in an endocrine manner mediated by CCK-B/gastrin receptors in tumor cells(Watson et al. 1993).G17 is particularly implicated in stimulating the growth of colorectal adenocarcinomas due to a possible increased affinity for the CCK-B/gastrin receptors on the tumor cells, as compared to other gastrin hormone species (Rehfeld 1972 and 1993). The CCK-B/gastrin receptors were found to be expressed in a high affinity form on 56.7% of human primary colorectal tumors (Upp et al. 1989).

Numerous studies have shown that, in addition to being able to respond to exogenous endocrine gastrin, human gastric and colorectal tumors produce gastrin and its precursors (Ciccotosto et al., 1995; Finley et al., 1993; Kochman et al., 1992; Nemeth et al., 1993; Van Solinge et al.,1993),thus effecting an autocrine growth stimulatory pathway.Gastrin production in tumor cells differs from that of endocrine G cells.Specifically,those tumor cells contain a high proportion of the precursor progastrin along with a lower concentration of mature peptides.This abnormal ratio is postulated to be due to constitutive unregulated release of gastrin combined with a limited activity of peptidylglycine α-amidating monooxygenase (Ciccotosto et al., 1995; Kelly, 1985).Thus, the unregulated release of gastrin leads to the abnormal production and secretion of different molecular forms of the hormone.Specifically,colon carcinoma cells do not efficiently process progastrin resulting in less conversion of precursor gastrin to the mature peptides and,thus,produce mostly incomplete or aberrant gastrins,(Dickinson 1993 and Rehfeld et al. 1993). In addition, the increased gastrin level in colorectal tumors is, in part, attributed to the aberrant expression of the gastrin gene in the colorectal tumor cells(Hoosein et al.1990, Baldwin et al. 1992 and Finley et al.1993).Gastrin-like peptides have been identified in such cells(Hoosein et al.1988,Watson et al. 1991 and Finley et al.1993),and were confirmed to be precursor gastrin species (Van-Solinge et al. 1993 and Nemeth et al. 1993).

The presence of amidated-G17 (G17-NH_2) in some colorectal cancers(Ciccotosto et al., 1995; Van Solinge et al., 1993) demonstrates that some tumors retain an intact processing

pathway, as gastrin amidation only occurs in secretory granules (Varro et al., 1994). Endogenously produced gastrin also acts as an autocrine growth factor, since the basal growth of a colorectal cell line was shown to be inhibited by an anti-gastrin antibody (Hoosein et al., 1988). This was confirmed in a second study in which Northern blot analysis revealed gastrin mRNA in the same cell lines and radioimmunoassay revealed gastrin-like immunoreactivity in cell culture supernatant (Hoosein et al., 1990). Gastrin peptides also possess paracrine roles (Watson et al., 1991b)which was confirmed(Finley et al.,1993)in experiments showing gastrin immunoreactivity more predominant in subpopulations of malignant colorectal mucosal cells.

When G17 binds to its receptor a G17/receptor complex is formed which stimulates cell growth by way of secondary messengers for regulating cell function (Ullrich et al. 1990). The binding of G17 to the CCK- B/gastrin receptor leads to activation of phosphatidylinositol breakdown, the protein kinase C activation with a resultant increase in intracellular calcium ion concentration, and the induction of *c-fos* and *c-jun* protooncogenes via the mitogen-activated protein kinase, which has been implicated in the regulation of cell proliferation (Tadisco et al. 1995). Additionally, gastrin binding to the CCK-B/gastrin receptor has been associated with the subsequent increase in phosphorylation by a tyrosine kinase, the pp125FADK(focal adhesion kinase),which may also have a role in the transmission of mitogenic signals (Tanaguchi et al. 1994).

Colorectal cancer remains a formidable disease to treat, as only minor improvements in survival have been obtained in recent years.Surgery is an effective treatment of the primary disease, but it is ineffectual against residual occult disease,which is frequently present. Radiation therapy post-surgery is generally recommended for patients with rectal cancers to reduce the risks of recurrence of the disease.Chemotherapy with 5-fluorouracil(5-FU) has been the most traditional effective therapy following surgery in patients with more advanced colorectal cancers.However,5-FU therapy has been shown to be only of marginal benefit to the patient,since 5-FU is highly toxic and the therapy is costly and does not appear, alone or in combination with other cytotoxic drugs, to significantly prolong survival. In most instances, occult or inoperable colorectal tumors do not respond well to chemotherapy or radiation, and new treatments are needed to supplement present procedures.

Recently, several studies have shown that adjuvant combination chemotherapy with 5-FU and Leucovorin improves the efficacy of 5-FU in patients with advanced colorectal cancer. Leucovorin is a folic acid derivative, also known as folinic acid, Citrovorum factor, or 5-formyl-5,6,7,8,-tetrahydrofolic acid. The studies show that in Dukes' stage C patients, 5-

FIG. 1 depicts a graph showing a time scale of serum antibody titers after immunization of rats immunized with 500 μg/ml of rat anti-G17 (1-9)-DT immunogen.

FIG. 2 depicts a graph showing the effects of 30 mg/kg dose of 5-FU/leucovorin treatment on the anti-G17(1-9) antibody titers obtained in rats immunized with the immunogen of the invention.

FIG. 3 depicts a Scatchard plot showing the effects of treatment cycles of 30 mg/kg of 5-FU/leucovorin on the mean white blood cell counts in BDIX rats.

FIG. 4 depicts a bar graph showing the median tumor weight of untreated, anti-G17(1-9) DT-treated and DT-treated rats.

FIG. 5 depicts a bar graph showing the median tumor weights of rats treated with 30mg/kg of 5-FU/leucovorin;30 mg/kg of 5-FU/leucovorin and DT immunogen;30 mg/kg of 5-FU/leucovorin and anti-G17(1-9)-DT;25 mg/kg of 5-FU/leucovorin and DT immunogen;25 mg/kg of 5-FU/leucovorin and anti-G17(1-9)DT; 20 mg/kg of 5-FU/leucovorin and DT immunogen; 20 mg/kg of 5-FU/leucovorin and anti-G17(1-9)DT; 12.5 mg/kg of 5-FU/leucovorin and DT immunogen; and 12.5 mg/kg of 5-FU/leucovorin and anti-G17(1-9)DT.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods of treating tumors, in particular those associated with gastrin-dependent colorectal cancer, with a combination therapy comprising immunizing a patient with an anti-G17 immunogen and treating the patient with chemotherapeutic agents,such as 5-FU and leucovorin.The anti-G17 immunization/5-FU-leucovorin combination therapy, surprisingly,has been found to be more effective than previous therapies in treating colorectal cancer. The chemotherapeutic agents useful in the combination therapy do not significantly inhibit anti-G17 antibody production in an immunized patient and lower doses of chemotherapeutic agents can be used for treating the tumor growth.In addition, the anti-G17 antibody titers produced by immunization are effective to neutralize all forms of G17 hormone.

In a preferred embodiment, the method comprises actively immunizing a patient afflicted with a gastrin-dependent colorectal cancer applying an anti-G17 immunogenic composition in conjunction with administering to the patient chemotherapeutic agents. Subsequent booster anti-G17 immunizations may be administered as required by the patient,as determined by analysis of the patient's serum anti-G17 antibody titers post-immunization,using

standard techniques and standard radiological assessments of the tumors. Anti-G17 immunization may also be provided to a patient prior to tumor surgery.

The anti-G17 immunogens comprise a natural or synthetic peptide fragment of the N-terminal amino acids of G17 as the immunomimic portion of the immunogen.This peptide fragment is conjugated to an immunogenic carrier such as Diphtheria toxoid (DT).In a preferred embodiment of this aspect of the invention, the anti-G17 immunogen comprises the amino-terminal amino acids of G17 from positions 1 through 9, having the amino acid sequence pyroGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu, conjugated to Diphtheria toxoid. Other suitable immunogenic protein carriers, include bovine serum albumin, keylimpet hemocyain, hemocyanin and tetanus toxoid.

The immunogens of the invention may also comprise an extension or a spacer peptide sequence suitable for projecting the immunomimic peptide away from the protein carrier and for enhancing its capacity to bind the lymphocyte receptors. A suitable spacer peptide sequence is the amino acid sequence SSPPPPC (SEQ ID NO.: 2 in the Sequence Listing). However, other spacer peptides would be suitable as well. In a preferred embodiment of this aspect of the invention, the preferred spacer sequence is attached to the carboxy-terminal end of the immunomimic peptide. The immunogens of the invention are produced by standard techniques and are disclosed in U.S. Pat. Nos. 5,023,077; 5,468,494; 5,607,676; 5,609,870; 5,688,506 and 5,662,702, the disclosures of which are hereby incorporated by reference. Following immunization, the immunogens of the invention produce high affinity, neutralizing antibodies for inhibiting the effects of G17 in its mature and precursor forms on tumor growth in immunized animals.The anti-G17 antibodies produced bind and neutralize mature and precursor G17, thereby preventing the binding of G17 to the receptors on tumor cells and ultimately inhibiting tumor cell growth. The immunogens raise antibodies which neutralize both the carboxy-amidated and glycine-extended G17, and show no cross-reactivity with G34 or CCK.

The compositions in which the immunogens for active immunization are administered for the treatment of gastrin-dependent tumors in patients may be in a variety of forms. These include, for example, solid, semi-solid and liquid dosage forms, such as powders, liquid solutions, suspensions, suppositories, and injectable and infusible solutions.The preferred form depends on the intended mode of administration and therapeutic applications. The compositions comprise the present immunogens and suitable pharmaceutically acceptable components, and may include other medicinal agents, carriers, adjuvants excipients, etc.,which can be mixed using standard procedures. Preferably, the compositions are in the form of unit

Example 6

Treatment of human colon cancer patients with a combination therapy of 5-FU/Leucovorin and anti-G17 (1-9)-DT.

Anti-G17(1-9)-DT immunization alone has previously been shown to be a valuable and safe therapeutic option in the treatment of gastrin-dependent cancer. The present combinations of anti-G17 immunogens with 5-FU/Leucovorin enhance the effectiveness of cancer treatment,in particular colon cancer treatment,and the possible reduction in the dosage of the chemotherapeutic agent required in the combination should reduce the deleterious cytotoxic side effects of any of the chemotherapeutic agents now in use. The present combinations of an immunogen with chemotherapeutic agents may also be useful as a second-line therapy in patients who do not respond to chemotherapy alone.

Human colorectal tumor or colon cancer patients are treated with a combination of chemotherapy and immunotherapy.

Specifically,for patients with gastrin responsive colorectal tumors or colon cancer can be treated with concomitant administration of 5-FU/Leucovorin and an anti-G17 immunogen composition or anti-G17 antibodies.

In particular, the preferred immunotherapy provides an immunogenic composition comprising an aminoterminal G17 (1-9) peptide: DT conjugate in a pharmaceutically acceptable carrier which may include an adjuvant to further stimulate the immune response.

The preferred immunotherapeutic regimen can start before, during or after the chemotherapy course depending on clinical considerations. For example, in a patient with a large tumor burden it may be advantageous to start with several cycles of chemotherapy to reduce the tumor bulk and then start with immunotherapy.

Alternatively, in a patient with a small tumor burden or after curative surgery, immunotherapy can be started before or during chemotherapy.

The active immunization dose can range between 300 μg up to 1200 μg of the anti-G17 immunogen, depending on the immune status of the patient (or the capacity of an immune response). The injection intervals can be on days 1, 7 and 14, or days 1, 14 and 21,or days 1, 14, then 28 and 56. All the schedules can result in similar antibody titers. The accelerated schedules of immunization provide the possibility of earlier onset of immune response.

The preferred method of the anti-gastrin therapy provides that a booster is administered every 6 months after the initial immunization period, regardless of which protocol is used.

Yet another preferred method for the effective neutralization of G17, Gly G17 and G17 NH_2 provides passive immunization with anti-G17 antibodies, preferably in purified form.More specifically, the inoculation of 10-1000 μg anti-G17(1-9) antibodies is administered before, during and/or after the chemotherapy cycles for the control of gastrin activities. The passive immunization can be administered daily, weekly or biweekly. Other protocols can be followed depending on the effectiveness of the treatment.

A further combination of treatment provides for an initial passive immunization before and/or during the first cycle of chemotherapy followed by active immunization as described above.

Many chemotherapy regimens are in use. These art recognized regimens, although not described herein, are not excluded from the combination treatment according to this invention.One preferred chemotherapy regimen provides for 5-FU i.v. bolus of 425 mg/m^2 with i.v. infusion of Leucovorin (folic acid, FA, 20 mg/m^2) for 1-5 days per period up to 4 weeks.

Another preferred regimen provides for 200 mg/m^2 FA over a period of 2h, followed by 5-FU i.v. boles of 400 mg/m^2 + 5-FU of 600 mg/m^2 over 22 hours 1 or 2 days in a 2-week period.

Yet another preferred regimen provides for continuous infusion of 5-FU at 250-300 mg/m^2 day continuous i.v. for 4-6 weeks, followed by 2 weeks rest.